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(94) Method for the preparation of a fermented milk product.

(97) The invention relates to a method for the preparation of a fermented milk product, such as yoghurt, wherein milk is incubated with a thermophilic Lactobacillus, followed by destroying of the Lactobacillus and optionally adding non-incubated milk, and wherein the milk is then incubated with a Streptococcus.

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The invention relates to a method for the preparation of a fermented milk product by fermentation of milk with at least one bacterium of the genus *Lactobacillus*, in particular the species *L. delbrueckii*, subspecies *bulgaricus*, and one bacterium of the genus *Streptococcus*, in particular of the species *S. thermophilus*.

5 It is known that a series of products which are very attractive to the consumer can be obtained by fermentation of milk with microorganisms. A review of the prior art is given in Bulletin of the International Dairy Federation No. 227/1988. The large number of suitable species of microorganisms offers the possibility of a wide variation in texture and in the taste of the end product; the technique employed also has a great influence on the texture. A common characteristic is that the product contains live microorganisms.

10 Many of these products contain, optionally in addition to other microorganisms, a combination of *Lactobacillus delbrueckii* subspecies *bulgaricus*, and *Streptococcus thermophilus*. This combination forms the basis for the preparation of yoghurt-like products. Proto-cooperation occurs in this preparation: if these species are cultured together they stimulate one another's growth. Together they provide the characteristic texture and taste of yoghurt. The customary culture temperature is between 32 and 45°C; the inoculation percentages vary from 0.025 to 5%.

However, a problem arises in the case of fermentation with said cooperative combination: as the result of the continuing formation of acid and the progressive degradation of protein as a consequence of the presence of the *Lactobacillus* a sour and/or bitter taste can develop when the product is stored. The *Lactobacillus* is, however, indispensable because of its contribution to the flavour and its production of compounds which stimulate the growth of the *Streptococcus*; it is precisely because of the cooperation that a sufficiently rapid formation of acid is achieved which is important for the texture of the product.

Attempts have been made to solve this problem by pasteurising the product after preparation; however, it is found that an adequate texture can then be maintained only with the aid of added thickeners (Zuivelzicht 38 (1982) 852-854). Of course, the product then also no longer has the characteristic of the presence of live microorganisms in the product ready for consumption.

20 Attempts have also been made to arrive at a solution to the problem by preparing a product such as yoghurt without the *Lactobacillus* (J. Dairy Research 49 (1982) 147). In order to compensate for the lack of metabolites of the *Lactobacillus*, which results in a poor growth of the *Streptococcus*, growth stimulants such as hydrolysed casein are then added. However, these stimulants differ from the protein fragments which are formed as a result of the action of the *Lactobacillus*; consequently, the contribution of the *Lactobacillus* to the taste is missing and, in respect of the taste, the products can then also be differentiated from those products in which the *Lactobacillus* and the *Streptococcus* have been grown together. Moreover, there is a desire in the foodstuffs industry to avoid as far as possible additives which are obtained by chemical processing.

30 In European Patent EP 0,148,299 a method is described in which *Lactobacilli* are added, but only after the acidification by the *Streptococcus* in the presence of stimulants, which are not described in more detail, has gone to completion. In this case also there can be no question of proto-cooperation.

A solution to the problem of continuing acidification and progressive protein degradation due to the presence of the *Lactobacillus* is given in European Patent Application EP-A 0,322,010. According to the method described in this publication, the *Lactobacillus* and the *Streptococcus* are cultured in a fermenter, separated from one another by a semipermeable membrane; free exchange of substances with a relatively low molecular weight takes place between the two compartments. The product which is tapped from the compartment which contains the *Streptococcus* is found to be fully comparable in respect of taste and texture with the fresh product prepared in the traditional way. However, a disadvantage of this method is that special equipment, which is not available in traditional dairy companies, is required to carry it out.

45 It has now been found that it is possible to prepare a product such as yoghurt which is fully comparable with the corresponding product prepared in the traditional way, without pasteurising the product and without the use of equipment other than the conventional dairy equipment, by inoculating milk with a thermophilic *Lactobacillus*, incubating and then treating it in such a way that the *Lactobacillus* is destroyed and then using the product as a substrate for at least the *Streptococcus*.

50 In this context, milk must be understood as meaning any raw material suitable for the preparation of fermented milk products; primarily cows' milk, but also milk from other mammals, such as horses, sheep or goats. The milk may be full-fat, but also skimmed or partially skimmed; in some cases constituents such as water or casein will have been removed from the milk, but milk constituents - in particular in the form of ultrafiltration retentate - may also have been added.

A thermophilic *Lactobacillus* is understood to be a *Lactobacillus* having its optimum growth at temperatures above about 30°C.

The invention relates to the preparation of products in which the proto-cooperation between a thermophilic lactobacillus and a streptococcus plays a role. Usually the lactobacillus used will be *Lactobacillus delbrueckii*, subspecies *bulgaricus*, and the streptococcus *Streptococcus thermophilus*. If desired, the *Lactobacillus delbrueckii* subspecies *bulgaricus*, can be replaced, for example by *Lactobacillus helveticus*, which likewise forms a proto-cooperative combination with *Streptococcus thermophilus*. Moreover, the invention is not restricted to the preparation of yoghurt but is also applicable to the preparation of products in which, in addition to the proto-cooperative combination, yet further microorganisms play a role. Known examples of the said further microorganisms are *Bifidobacterium* species and *Lactobacillus* species such as *L. casei* and *L. acidophilus*.

In the dairy industry a differentiation is made in principle between two types of yoghurt-like products; the stirred product and the set product. Thus, stirred yoghurt is obtained by incubating milk in a tank with the necessary bacterial culture, then stirring until a smooth, homogeneous product has been obtained and packaging this product, while set yoghurt is prepared by incubating the inoculated milk in the packaging. When the method according to the invention is used, the milk inoculated with the streptococcus will first be incubated, then stirred until smooth and finally packaged in order to obtain stirred yoghurt, while the milk inoculated with the streptococcus will be incubated in the final packaging in order to obtain set yoghurt.

The method according to the invention can be represented as a number of successive steps:

a. A culture of a lactobacillus, in particular *L. delbrueckii* subspecies *bulgaricus*, is inoculated into the milk and cultured. The percentage inoculation and the culture temperature are those which are customary in the traditional preparation of yoghurt: 0.025 - 5% and 32-45 °C respectively; preferably, however, the percentage inoculation is between 2 and 3% and the culture temperature is between 40 and 45 °C.

b. The fully grown lactobacillus culture is heated in such a way that the bacteria present are destroyed. Because it is important that the heated suspension not only contains no living bacteria but also has little or no enzymatic activity, it is preferred to choose a temperature and time combination for this step which has at least the same effect as heating for 1 minute at 85 °C.

c. A culture of *S. thermophilus* is then inoculated into the suspension of destroyed lactobacilli, which is optionally mixed with non-incubated milk, and incubated. In this step also the percentage inoculations and culture temperatures customary in the traditional preparation of yoghurt are employed. The incubation is continued until the pH value desired for the particular product has been reached. For the preparation of stirred yoghurt a suitable procedure is, after inoculating with *S. thermophilus* culture, to culture at 37 °C until a pH value of 4.4 is reached.

d. When the product has reached the desired pH value, the product is cooled, stirred if necessary and, if desired, mixed with additives such as fruit.

The suspension of destroyed lactobacilli which is obtained according to steps a and b can be used as such as the substrate for *S. thermophilus* and any other microorganisms; preferably, however, before inoculation with the streptococcus, the suspension is mixed with non-incubated, pasteurised milk. Although growth stimulation of the streptococcus is already discernible with a mixing ratio of 1 part of suspension to 200 parts of non-incubated milk, as can be seen from Table 1, mixing ratios of higher than 1 to 50 are to be preferred.

TABLE 1

Effect of mixing ratio of milk incubated with lactobacillus to non-incubated milk on the rate of acidification of streptococcus culture

Lactobacillus culture (ml)	Non-incubated milk (ml)	Rate of acidification (-ΔpH/6 hours)
0	2000	0.70
10	2000	1.00
20	2000	1.10
40	2000	1.46
100	2000	1.94

Instead of using the lactobacillus culture as such, the suspension of destroyed lactobacilli can also be first dried; methods for drying material of this type are known to those skilled in the art. Thus, the suspension can first be concentrated by evaporation and then dried by spray-drying. The material obtained in this way can then, independently of the location of the production thereof, be suspended as powder in milk in order to prepare a substrate for the streptococcus. Preferably, with this procedure, amounts of between 1 and 15 g of the dried material per litre of milk are used; for a mixing ratio of about 0.5 g of powder to 1 litre of milk stimulation of the growth of the streptococcus is, incidentally, already found to occur.

Although it is possible to apply the method according to the invention by using the relatively highly acid suspension of destroyed lactobacilli mixed with milk as substrate for the streptococcus, it is preferred to bring the lactobacillus culture to a pH value of at least 5.8 during or after culture. The culture method which is known as "neutral culture" may be used for this purpose: if the pH has fallen to, for example, 5.8 during the incubation of the lactobacillus culture, this value is maintained during the further incubation by adding neutralising agents such as ammonia, dilute alkali or lime water. After the incubation is complete, the pH of the suspension can, if desired, be brought to the initial value using one of the said agents.

Formate stimulates the growth of *L. delbrueckii* subspecies *bulgaricus* (Th.E. Galesloot, F. Hassing and H.A. Veringa, *Neth. Milk Dairy J.* 22 (1968) 50-63). Preferably, the formate content is above 15 mg per kg of milk; if necessary, the content can be brought above the said value by adding formate.

Growth stimulation such as that caused by the presence of formate can also be obtained by subjecting the milk, prior to inoculation with the lactobacillus, to a heat treatment which at least corresponds to heating at 110° C for 10 minutes.

Of course, in the culture of the lactobacillus use can be made of the abovementioned stimulation of the lactobacillus growth by the streptococcus. In this case, inoculation and culture in the abovementioned step a is with both *L. delbrueckii* subspecies *bulgaricus*, and *S. thermophilus* for a time which corresponds to or is shorter than that for the traditional preparation of yoghurt. In step b all bacteria are then destroyed and, optionally after drying and adding non-incubated milk, the material thus obtained is inoculated with *S. thermophilus* (steps c and d).

The culture time is found to influence the organoleptic quality of the end product. A relatively short culture time, for example 38 hours at 37° C, is sufficient to produce a good texture of the end product, but only on prolonged incubation, for example 70 hours or longer at 37° C, is the end product found to contain more acetaldehyde, which results in a clear intensification of the flavour of the end product.

All products for which the proto-cooperation of *L. delbrueckii* subspecies *bulgaricus*, or *L. helveticus* with *S. thermophilus* plays a role in the production can be prepared by the present method. Not only products such as stirred yoghurt or set yoghurt, in which exclusively the said species typify the product, but also products in which microorganisms other than *S. thermophilus*, such as *L. acidophilus* or *Bifidobacterium*

bilidum, also play a role can be prepared using the method according to the invention. Thus, it is very attractive to eliminate the adverse effect of a growing *L. delbrueckii* subspecies *bulgaricus*, on the growth of *L. acidophilus* (J. Food Prot. 40 (1977) 760) by using the method according to the invention in the preparation of acidophilus yoghurt by mixing a suspension of destroyed lactobacilli with milk and inoculating and incubating this mixture with *S. thermophilus* and *L. acidophilus*.

The invention is illustrated by the following examples.

EXAMPLE I

2000 ml of full-fat milk were kept at 110 °C for 10 minutes, then cooled to 45 °C and inoculated with 2.5% (v/v) fully grown culture of *Lactobacillus delbrueckii* subspecies *bulgaricus*, strain Ib (CBS No. 903.87). The mixture was incubated at 45 °C until the pH had reached a value of 5.7 (time taken about 3 hours). The mixture was then brought to the original pH value of 6.5 by adding a solution of NaOH (1 molar) and heated at 85 °C for 1 minute.

After cooling the mixture to 37 °C, 2.5% (v/v) of a fully grown culture of *Streptococcus thermophilus* strain Sts (CBS No. 904.87) were added to the mixture and this culture was incubated at 37 °C until a pH value of 4.4 was reached (time taken about 14 hours). The product was stirred until a smooth mass was obtained and cooled to 7 °C. The product had a texture and flavour fully comparable with those of a yoghurt prepared by the traditional method; the pH value was 4.3.

The product was then stored for 14 days at 7 °C; at the end of this period the pH was found to be 4.2.

EXAMPLE II

2000 ml of full-fat milk were kept at 100 °C for 30 minutes, then cooled to 37 °C and inoculated with 2.5% (v/v) of a fully grown culture of *Lactobacillus delbrueckii* subspecies *bulgaricus*, strain Ib. The mixture was incubated at 37 °C; when the pH had reached a value of 5.8, this value was maintained during further culture by adding a mixture of NH₃ (5% m/v) and NaHCO₃ (20% m/v) in water. After a total culture time of 142 hours, the mixture was heated at 85 °C for 1 minute and then cooled. 100 ml of the mixture thus obtained were added to 2000 ml of full-fat, highly pasteurised (5 min/85 °C) milk; the mixture was then inoculated with 2.5% (v/v) of a fully grown culture of *Streptococcus thermophilus* strain Sts, after which the mixture was incubated at 37 °C until a pH value of 4.4 was reached (time taken about 8 hours). The product was stirred until a smooth mass was obtained and cooled to 10 °C. The texture and flavour of the fresh, cooled product were fully comparable with those of a yoghurt prepared by the traditional method. The pH of the fresh product was 4.3.

The product was then stored for 14 days at 7 °C; at the end of this period the pH was found to be 4.2.

EXAMPLE III (comparative example)

For comparison, yoghurt was prepared in the manner customary in the Netherlands dairy industry.

2000 ml of full-fat milk, which had been heated at 85 °C for 5 minutes, were inoculated, after cooling to 32 °C, with 0.025% (v/v) of a mixture of the commercially available yoghurt strains Ist and Rr (mixing ratio 1:1) (these strains are obtainable from Coöperatieve Stremsele- en Kleursefabriek, Leeuwarden). The mixture was incubated at 32 °C until the pH had reached a value of 4.4 (time taken about 15 hours). The product was then stirred until a smooth mass was obtained and cooled to 10 °C. The pH of the fresh, cooled product was 4.3.

The product was then stored for 14 days at 7 °C; during this period the pH fell to a value of 3.9.

Claims

- Method for the preparation of a fermented milk product by bacterial fermentation of milk with at least one thermophilic lactobacillus and one streptococcus, characterised in that milk is inoculated with at least the lactobacillus and incubated, and the incubated milk is treated in such a way that the lactobacillus is destroyed and is then inoculated with at least the streptococcus and incubated.
- Method according to Claim 1, characterised in that the milk incubated with the lactobacillus is mixed with non-incubated milk after destroying the lactobacillus.
- Method according to Claim 2, characterised in that one part of incubated milk is mixed with 0.1-50 parts

of non-incubated milk.

4. Method according to Claim 2 or 3, characterised in that the milk incubated with the lactobacillus is dried, after destroying the lactobacillus, before being mixed with the non-incubated milk.
5. Method according to one of Claims 1 - 4, characterised in that the pH of the milk inoculated with the lactobacillus is brought to a value of at least 5.8 during or after incubation.
6. Method according to one of Claims 1 - 5, characterised in that the milk which is inoculated with the lactobacillus contains at least 15 mg of formate per kg.
7. Method according to one of Claims 1 - 6, characterised in that the milk has been subjected to a temperature treatment which at least corresponds to heating for 10 minutes at 100°C before said milk is inoculated with the lactobacillus.
8. Method according to one of Claims 1 - 7, characterised in that the lactobacillus used is Lactobacillus delbrueckii subspecies bulgaricus, and the streptococcus used is Streptococcus thermophilus.
9. Fermented milk product which contains no or virtually no live lactobacilli and contains streptococci which are live as well as material which originates from dead lactobacilli and may have been converted by streptococci.
10. Fermented milk product prepared in accordance with the method of one of Claims 1 - 8.



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EUROPEAN SEARCH REPORT

Application Number

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
X	JOURNAL OF FOOD PROTECTION vol. 40, no. 6, June 1977, Madison, Wisconsin, US pages 406 - 410; M.A. COUSIN & al.: "Lactic acid production by Streptococcus lactis and Streptococcus cremoris in milk precultured with psychrotrophic bacteria" * pages 1 - 2 * * figure 1 *	1, 9, 10	A23C9/123
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X	FR-A-2197521 (UNILEVER) * page 1, lines 26 - 32 * * page 6, line 35 - page 7, line 6 * * page 8, lines 18 - 21 * * example 6 *	1, 10	
Y, D	NETHERLAND MILK AND DAIRY JOURNAL vol. 22, 1968, Amsterdam, NL pages 50 - 63; T.E. GALESLOOT & al.: "Syntiosis in yoghurt (I). Stimulation of Lactobacillus bulgaricus by a factor produced by Streptococcus thermophilus" * page 52, paragraph 2.7 * * page 53, paragraph 3.1 * * page 56; figure 1 * * page 58; figure 2 * * page 58, paragraph 3.7 * * page 59 *	1, 5-10	TECHNICAL FIELDS SEARCHED (Int. Cl.5) A23C
Y	NETHERLAND MILK AND DAIRY JOURNAL vol. 4, 1950, Amsterdam, NL pages 209 - 224; J.W. PETTE & al.: "Yoghurt: II. Groefactoren voor Streptococcus thermophilus" * pages 212-216, paragraph III *	1, 5-10	
Y	US-A-3985901 (C. G. BARBERAN) * claims 1-4 * * figure * * column 5, line 25 - column 8, line 45 *	4	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 28 MAY 1991	Examiner VUILLIARD V.M.L.
CATEGORY OF CITED DOCUMENTS		I : theory or principle underlying the invention F : earlier patent document, but published on, or after the filing date D : document cited in the application I : document cited for other reasons	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		A : member of the same patent family, corresponding document	

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DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
The present search report has been drawn up for all claims			
Place of search THE HAGUE	Date of completion of the search 28 MAY 1991	Examiner VUILLAMY V.M.L.	
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention F : earlier patent document, but published on, or after the filing date I1 : document cited in the application I : document cited for other reasons	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background Q : non-written disclosure P : intermediate document		A : member of the same patent family, corresponding document	